



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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Memorandum

Date August 11, 1989
From Deputy Director, Division of Toxicological Review and Evaluation, HFF-152
Subject Evaluation of Data Concerning Possible Mechanism(s) Mediating Rat Thyroid Tumorigenesis by FD&C Red No. 3
To Ronald Loentzen, Ph.D.
Assistant to Director for Carcinogenicity Assessment, HFF-100

Below is reviewed data derived from a number of studies with FD&C Red No. 3. This data survey is not intended to be comprehensive with respect to all studies and adverse effects found, but focuses on those data that may influence the Cancer Assessment Committee's decision whether FD&C Red No. 3 mediates thyroid tumorigenesis in the rat via a primary (direct) or a secondary (indirect) mechanism as is hypothesized by the sponsors of FD&C Red No. 3. This review of potential carcinogenic mechanisms for FD&C Red No. 3 is necessitated by an upcoming decision whether to terminate the provisional listing for FD&C Red No. 3.

INTRODUCTION

According to a recently developed International Life Sciences Institute (ILSI) review (1986), FD&C Red No. 3 has at least 43 different reference names in the world literature. The most common nomenclature used in domestic scientific literature is erythrosine or FD&C Red No. 3. In its permanent listing under 21 CFR 74.303, FD&C Red No. 3 is referred to as the monohydrate of 9(o-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3H-xanthen-3-one, disodium salt with smaller amounts of lower iodinated fluoresceins (see Figure 1 for structure). The structures for T_1 and T_2 are also listed in Figure 2 for comparative purposes. FD&C Red No. 3 is provisionally listed in 21 CFR 81.1 for cosmetic coloring purposes.

FD&C Red No. 3 is used in dietary supplements, confectionary products, dairy products, cherries, jellies, jams, dessert powders, canned fruit and vegetable products, fish products, condiment sauces and bakery goods. For the purposes of cosmetic coloring, FD&C Red No. 3 is used in creams and lotions, face and body powders, dry, liquid and cream rouges, cake makeup, shampoo, bath preparations, perfume, antiperspirants and shaving preparations.

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ESTIMATES OF EXPOSURE

The acceptable daily intake as listed by JECFA and EEC are 0-0.05 mg/kg (33rd Report, 1988) and 0-1.25 mg/kg (14th Series, 1983), respectively. In the U.S., it is presently covered by GMP regulations (21 CFR 74.303). The current exposure to FD&C Red No. 3 and its lakes (based on Food and Color Additives Review Section memorandum, December 11, 1986 to Division of Food and Color Additives) from dietary ingestion is estimated to be 9.0 mg/day for young children (ages 2-5) and for other age groups (ages 5 plus). On a weight basis, however, the exposure is estimated to be 600 ug/kg for children (ages 2-5) and 150 ug/kg for other groups (ages 2 plus).

Consumers may also be exposed to FD&C Red No. 3 from ingested drugs or dietary supplements (17-50 ug/kg derived from 60 kg body weight). In patients consuming drug syrups, the combined short term exposure to FD&C Red No. 3 may increase to almost 2-fold the levels estimated for chronic exposure, to approximately 20 mg/day. Thus, children in the 2-5 years category could receive short term exposures of 1300 ug/kg/day.

LONGTERM TOXICOLOGICAL STUDIES

The results of a longterm mouse feeding study were submitted to the Center for Food Safety and Applied Nutrition (CFSAN) on August 31, 1981 (International Research and Development Corporation or IRDC Study No. 410-005). In this study, 60 male and 60 female Charles River CD-1 mice received FD&C Red No. 3 administered in the diet in concentrations of 0, 0, 0.3, 1.0, and 3.0% for 104 weeks. While the urine of the high dose group mice was orange and the feces red, there were no hematological values in any dose group that were adversely affected in any systematic manner. In addition, there were no macroscopic lesions attributable to compound administration and microscopic examinations revealed no neoplastic lesions at any FD&C Red No. 3 dose level that were in excess of those demonstrated by the control animals.

The data from a longterm rat feeding study (IRDC Study No. 410-002) were submitted to CFSAN August 31, 1981. In this study, Charles River CD-1 rats were exposed in utero to FD&C Red No. 3. In F₀ animals FD&C Red No. 3 was administered in doses of 0, 0.1, 0.5, and 1.0% in the diet. F₁ pups (70 males and 70 females/group) were randomly selected and given FD&C Red No. 3 in the same dose levels. An interim sacrifice of 10 animals/sex/group was conducted at 12 months. The balance of the experimental animals (60M & 60F) were continued on their exposure to FD&C Red No. 3 until death or terminal sacrifice at Week 127 for females and Week 129 for males.

The incidences of masses were similar for both treated and control rats. Urine color was light or dark orange in color. Food consumption was only slightly higher in treated animals than in controls. Body weights were similar for control and treated animals from Week 26 to the end of the study. Mean thyroid weight and thyroid-to-body weight ratios were higher in the female 0.5 and 1.0% dose levels when compared to controls. Microscopic examination for neoplastic lesions revealed higher rates of

thyroid follicular adenomas in the 1.0% dose female and 0.5% dose male rats than controls (Table 1 and 2). In addition, a statistical analysis of combined, thyroid follicular adenomas and carcinomas in the male rats receiving 0.1, 0.5 and 1% doses of FD&C Red No. 3 compared to controls showed a statistically significant increase, respectively $P = 0.016$, 0.0007 and 0.03 (Memorandum from DM, J.K. Lee to B. Jackson, April 9, 1984).

A second longterm rat study was initiated at the request of the FDA. This study (IRDC Study No. 410-011) was submitted to CFSAN on August 2, 1982. It was conducted following the same in utero design as the previously reported study, No. 410-002. Charles River CD-1 rats (120 M and 130 F) were exposed to 0 and 4.0% FD&C Red No. 3 for 64 days, then at age 106 days they were mated. F₁ males and females (70 of each) were randomly selected and assigned to 2¹ groups. The males and females remained on diet for 125 and 122 weeks, respectively.

During the study, there was a statistically significant increase in food consumption by treated males and females compared to the control group ($p < .01$). There were no consistent or systematic changes in the hematology or biochemical data. The mean absolute and relative thyroid weights were increased in the 4.0% group compared to controls.

There was a greater incidence of enlarged thyroids in the treated males upon macroscopic examination (Tables 3 and 4) and this finding was correlated with an increased incidence of thyroid tumors following microscopic examination of the treated males at terminal sacrifice. While increased incidences of adrenal pheochromocytoma/medullary adenoma, testicular interstitial cell adenoma in males and adenomas in livers and uterine polyps in the treated females were reported in the study (Table 5), when the incidence rates were compared to spontaneous rates of tumor occurrence in most of these organ sites in this particular strain, the main tumorigenic finding was thyroid follicular adenomas (Memoranda of Conferences of CAC Committee for December 13, 1982, October 25 and November 10, 1983).

The comparative rates of thyroid follicular adenomas (Table 6) in the two groups of male rats were: Controls 1/68 (1.5%) and 4.0% 14/68 (20.6%). A prevalence analysis of these rates of occurrence revealed that the male treatment group suffered a significantly increased incidence ($p=0.005$) of thyroid follicular adenomas compared to controls (DM memorandum, J.K. Lee to M. Mack, December 9, 1982). Furthermore, there was a statistically significant increase in the combined incidence of thyroid follicular cell adenomas and carcinomas 18/68 (26.5%) for the 4.0% group, compared to controls (2/68 or 2.9%) (memorandum from DM, J.K. Lee to P. Siu, August 2, 1983).

The morphological response of the rat thyroid to treatment with FD&C Red No. 3 at the one year interim sacrifice is of interest in this study. However, the diagnoses of the thyroid response at this interim sacrifice are inconsistent. In a published paper reviewing the results of these last two studies (IRDC Study No. 410-002 and 410-011), Borzelleca et al.

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(1987) report that in the 10 rats of each sex from each group sacrificed and necropsied at 1 year on test in both studies, there were no compound-related gross or microscopic changes. In a report by Dr. Charles Capen, dated September 27, 1982 (no vol.), a description of proliferative responses present at the 12-month interim and terminal sacrifices was given. Dr. Capen concludes that 92% (60/65) of the 4% FD&C Red No. 3 fed rats had hypertrophy and hyperplasia whereas only 5.8% of the control rats were similarly affected. It is not clear how the denominator for this conclusion is obtained as the table of thyroid incidence data on the next page gives the total number of animals examined as 69.

Each treatment group had 70 rats/sex assigned to it and 10 of these animals were examined at the 1 year interim sacrifice. In the Borzelleca et al. paper (1987) none of the interim sacrificed FD&C Red No. 3 treated rats were diagnosed as having thyroid follicular hyperplasia; however, in Dr. Capen's report even if one assumes that all of the thyroids of the terminally sacrificed animals had hyperplasia, it would mean that, at least, 5 of the 10 of the one year interim sacrificed animals were diagnosed as having thyroid hyperplasia.

In a very recent submission, CCMA submitted (CAP 96, Vol. 55, Appendix 2.1) data supplied by Dr. Charles Capen on these one year, interim sacrificed animals. According to this latter data, 9 of 10 rats demonstrated hyperplasia (Table 7).

This conclusion may be contrasted with the conclusions of DP (Memorandum from R. Moch to B.A. Jackson, May 27, 1983) in which the denominator indicates that all treated rats including the 1 year interim sacrificed animals were examined; however, from Appendix B of the DP, May 27, 1983 memorandum there is only one animal listed as an interim sacrifice animal with hyperplasia and it is listed as a control animal. On page 8 of this same memorandum, however, there is a discussion of Dr. Capen's September 27, 1982 report and reference to Dr. Capen's use of "another category of classification of follicular lesions; i.e., "Background Follicular Cell Response...." In any case, it is not clear what the comparative diagnostic criteria were for determination of thyroid hyperplasia on the part of Dr. Capen and DP.

SHORTTERM TOXICOLOGICAL STUDIES

Subsequent to the tumorigenic findings of the IRDC chronic rat study, the sponsors of FD&C Red No. 3, the Certified Colors Manufacturers Association (CCMA), developed the hypothesis that the thyroid tumors found following in utero and lifetime exposure of Charles River CD-1 rats to FD&C Red No. 3 were mediated by an indirect or "secondary" mechanism. It was assumed that if sufficient evidence could be developed to adequately support this secondary mechanism, it might be possible to discover a threshold dose of FD&C Red No. 3 that would not produce the changes that are theorized to lead to the expression of thyroid tumors. Below are reviewed a number of studies that have been sponsored by the CCMA and submitted to the FDA as evidence supporting a secondary mechanism of carcinogenesis by FD&C Red No. 3.

1. Feeding Studies:

FRI Study (Project No. CM-70r)

The CCMA submitted a 6-month feeding study to the FDA entitled, "An Endocrine Evaluation of the Thyroidal Effects of FD&C Red No. 3." This study (FRI Project No. CM-70r) was performed by the Primate Research Institute at New Mexico State University, Holloman Air Force Base, New Mexico. The objective of this study was to test whether the thyroid follicular tumors found in the IRDC chronic study were caused by an excess of iodine, as the iodide ion, resulting from either FD&C Red No. 3 contamination or due to FD&C Red No. 3 metabolism or caused by some other non-iodine-related property of the color itself.

The various substances, FD&C Red No. 3 (commercial grade), FD&C Red No. 3 (highly purified) and Sodium Iodine (analytic grade) were administered following admixture in the diet. Treatment groups were specified as follows:

<u>GROUP</u>	<u>TEST ARTICLE</u>
I - Control	No test substance in diet
II - Ethanol Control	Ethanol control - used for NaI solubilization in other groups
III - Commercial Color	FD&C Red No. 3 at 4% of diet
IV - Purified Color	FD&C Red No. 3 at 4% of diet
V - Sodium Iodide	NaI at 80 ug/gm of diet
VI - Purified Color and Sodium Iodide	FD&C Red No. 3 at 4% of diet plus NaI at 40 ug/gm of diet
VII - Purified Color and 2-Fold Sodium Iodide	FD&C Red No. 3 at 4% of diet plus NaI at 80 ug/gm of diet

Four hundred twenty Sprague Dawley rats from Charles River Laboratories were used in this study. The test substances listed above were added to the control diet of Purina Certified Rodent Chow No. 5002 and water was supplied ad libitum from the Holloman AFB water system.

Twenty animals from each of the 6 treatment groups listed above were randomly selected and sacrificed at Week 6, 13 and 21. These times were chosen to encompass times of expected endocrine change and morphological and hormonal changes were compared at the same time points.

Animals were observed clinically two times daily for pharmacological or physiological effects mediated by the test compounds. Body weights and food consumption were recorded weekly. All animals found dead or sacrificed according to schedule were subjected to gross necropsy and an examination of a full complement of tissues (33 organs and tissues). Organ weights of brain, pituitary, thymus, adrenals, liver, kidney, heart, spleen, ovaries, testes, and thyroids were determined after fixation in 10% buffered formalin.

Five animals/sex/dose were anesthetized with barbiturate and perfused with nitrate, heparin, isotonic saline, and phosphate-buffered 5% glutaraldehyde for the purposes of tissue fixation in preparation for electron microscopy. No description was provided of how the principal investigator selected the area of each thyroid that would be histologically analyzed.

In addition to the histological examination and analyses, blood and serum samples were collected and subjected to radioimmunoassay for TSH, T_3 , T_4 and protein bound iodine (PBI) at Week 3, 6, 9, 13, 17, and 21. Serum samples were analyzed at two time points: the "in-life" assay was conducted immediately after the blood was withdrawn from the test animals and a "serial" assay was done by collecting the blood from the animals taken at each time point and freezing it and then assaying it at the completion of the experiment.

Body weights decreased significantly ($P < .05$) in all treatment groups except NaI (given by itself), 80 ug/gm level. The decreases were time-related but not dose-related with respect to NaI. Food consumption levels were increased significantly ($p < .05$) in all but the same 80 ug/gm NaI group. The thyroid weights of males were increased significantly ($p < .0007$, see Division of Mathematics memorandum R.K. Chi to R. Lorentzen, May 24, 1984) when compared to controls. The mean values for thyroid weights were control 35.2 mg and the treated groups 43.5 mg, a 23.6% increase in average thyroid weights.

A comparison of the hormonal results of control versus treated male animals (see Division of Mathematics memorandum R.K. Chi to R. Lorentzen, May 24, 1984) indicated a significant effect for the "in-life" assay with TSH ($p < .041$) and T_4 ($p < .0001$) being elevated and T_3 being depressed compared to the control groups. The "serial" assay of these same serum samples, however, showed no elevation of TSH ($P = .316$) or depression of T_3 ($p = .316$), but the levels of T_4 ($P < .0001$) were still elevated compared to control groups.

In this same study, female rats were also tested and a statistically significant decrease was found in body weight ($p = .011$) but thyroid weights were similar between control and treated groups. Neither "in-life" nor "serial" assays results indicated a significant increase in TSH ($p = .161$ and $= .524$, see Division of Mathematics memorandum from R.K. Chi to R. Lorentzen, July 12, 1984) and T_3 levels ($p = .07$) were not depressed when compared to controls. There were significant increases ($p < .0001$) with regard to T_4 concentrations in both the "in-life" and "serial" assays. Also there was a significant decrease ($p < .0001$) in the levels of T_3 in the "serial" assay.

Clinical chemistry data demonstrated a decrease ($p < .05$) in triglycerides and an increase in free fatty acids, in all treatment groups, except the 80 ug NaI/gm diet group, when compared to control groups. PBI and plasma iodine levels were elevated in all treatment groups.

In a November 30, 1984 submission to the FDA (Vol. 36, p. 11338-11343), Dr. Charles Capen provided an ultrastructural evaluation of rat thyroid glands from the FRI study. He concluded that: electron microscopic evaluation of the thyroid glands from rats fed FD&C Red No. 3 demonstrated hypertrophy of follicular cells with increased development of synthetic and secretory organelles associated with the biosynthesis of thyroid hormones. These changes were interpreted as representing a mild-to-moderate stimulation of follicular cells and were consistent with the sustained elevation in serum T₄ in the treated rats. Moreover, lysosomal bodies in rats receiving FD&C Red No. 3 were larger, more irregular in shape, and more electron-dense than controls.

In a memorandum reviewing this information (B. A. Jackson, Color and Cosmetics Branch to M. Mack, Division of Food and Color Additives, January 18, 1985), DP concluded that the report of the ultrastructural findings was not useful in defining the toxic effects of FD&C Red No. 3. The material submitted was incomplete with no indication of how the samples were selected or prepared. This evaluation memorandum also concluded that while the ultrastructural changes in some cells can be associated with circulating hormone levels, these observations seem to add little to what is already known about the toxic effects of FD&C Red No. 3. Moreover, any conclusions about carcinogenicity based on the results of the ultrastructural study would be extremely tenuous because of the tremendous sampling problems inherent in electron microscopic examinations.

Later, in response to the criticism that no actual electron micrographs were available for review, CCMA provided these data (submission received April 5, 1985). In its evaluation, the Division of Pathology (B.A. Jackson to M. Mack, DFCA, January 13, 1986) concluded that the electron micrographs of follicular cells from thyroid glands of male and female rats fed the control diet (Group I) and those fed the 4% commercial FD&C Red No. 3 diet (Group XIII) revealed no conspicuous and consistent differences in the ultrastructural appearance of these organelles that could be ascribed to treatment. The organelles in the follicular cells, rough-surfaced endoplasmic reticulum, the Golgi complex, lysosomal bodies and microvilli, displayed similar degrees of development in both control and treated rats.

Hazleton Laboratories Study (No. 6145-101)

CCMA submitted the final report on this study, entitled "Seven-Month Feeding Study to Assess the Effect of FD&C Red No. 3 on Thyroid Function in Rats" to the FDA on November 5, 1984 (CAP 96, Vol. 37, p. 11624-12118). The study was conducted during the time period September 1, 1983 to April 18, 1984. The objective of this study was to determine the influence of seven months continuous exposure to FD&C Red No. 3 on thyroid function in rats. One hundred eighty Sprague-Dawley CD-I rats from Charles River Laboratories (Wilmington, Massachusetts) were randomly assigned to the following treatment groups: 0%, 0.25%, 0.5%, 1.0%, 2.0%, and 4.0% FD&C Red No. 3 added to the daily diet.

Body weights, food consumption and other in-life observations were recorded weekly. All animals were bled before initiation of the study, at monthly intervals thereafter and at the end of the study. Urine samples were collected from randomly selected animals during the fifth and seventh month, and fecal samples were collected at seven months for measurement of total iodine.

At the start of the seventh month and continuing for approximately one month, five randomly selected rats/sex/group received daily subcutaneous injections of T₃ (dose of 1.5 ug/100 gm body weight) and another five randomly selected rats/sex/group received daily subcutaneous injections of saline. The remaining five rats/sex/group continued to receive only FD&C Red No. 3 or control diet.

Diet was provided ad libitum and was composed of Purina Certified Rodent Chow No. 5002. Demineralized and sterilized water was dispensed on an ad libitum basis. The test compound, FD&C Red No. 3 (Lot No. AC4862), was administered in the diet. Samples from the dietary admixtures for the highest and lowest dose levels were analyzed for homogeneity and stability.

Animals were viewed two times daily for signs of morbidity, death or indications of toxic effects. Body weight and food consumption were measured weekly. Prior to the beginning of the study, monthly, and at the termination of the study animals were bled from the orbital sinus to collect samples for determinations of possible hormonal effects mediated by FD&C Red No. 3. Whole blood was frozen and shipped to the consultant laboratory specified by the sponsor.

All animals found dead or sacrificed according to schedule were subjected to gross necropsy and a full complement of 33 tissues was preserved in 10% buffered formalin. For animals dying during the study and for those sacrificed at the termination of the study, thyroid and pituitary glands were weighed and fixed or fixed and weighed, respectively.

Animals were healthy for the duration of the study and the few adverse physical findings observed were those normally suffered by all experimental animals or were related to the bleedings from the orbital sinus. Mean body weights for the 2% and 4% female dose groups were lower than the female control group ($p=.047$ and $p=.0004$, see Division of Mathematics memorandum to DFCA, J.K. Lee to M. Mack, January 24, 1985). Food consumption for the 2% and 4% male treatment groups were consistently higher than those for the controls; and for females, the 4% group ate more food than controls.

Mean values for total iodine in the urine collected at 5 months from both male and female 4% rats and from 0.5% and 4% males at 7 months were significantly higher ($p < .0001$) than controls (Division of Mathematics memorandum to DFCA, J.K. Lee to M. Mack, January 24, 1985). In addition, mean values for total iodine in the feces of 0.5% and 4% males were significantly higher ($p < .05$) than control males. There was no

significant difference in the mean absolute thyroid weights between the treated and control rats.

Results from the hormonal portion of this study were reported separately in a submission referred to as "Studies of the Effects of Chronic Erythrosine Feeding on Various Aspects of Thyroid Hormone Economy in Rats" received by the CFSAN on December 4, 1984 (CAP 96, Vol. 35, p. 11187) The data provided in that paper was analysed by the Division of Mathematics (J.A. Springer to P.M.L. Siu, March 20, 1986).

With regard to the male subjects there was no significant difference for TSH between groups. Serum T_4 levels of the 4% group were significantly elevated for the entire 6-month period. In addition, the T_4 for the 4% dose groups was significantly higher ($p < .003$) than the 0.5% dose level for Months 1, 2, 3, and 6. All groups' T_4 was significantly elevated at the one month sampling time compared to the baseline values. The serum T_3 levels for the 4% but not for the 0.5% dose group were significantly lower ($p = .037$) than controls values. In animals receiving the 4% dose of FD&C Red No. 3, the serum levels of rT_3 were clearly elevated (a 7-fold increase; $P < 0.0001$) for the duration of the study. The control and 0.5% serum levels of rT_3 were virtually the same. Values of serum rT_3 were undetectable in the animals that received injections of T_3 during the final month of the study.

The second portion of the hormonal results (in female rats) were reported in another submission entitled, "The Effects of Chronic Feeding of Erythrosine on Thyroid Hormone Economy in Female Rats" (Memorandum of Transmittal, DT to DM, September 26, 1985). A statistical analysis provided by DM (J.A. Springer to P.M.L. Siu, March 20, 1986) of the sponsor's findings indicated:

- There were no significant differences in TSH levels between the respective treatment groups.
- T_4 levels were significantly elevated ($p < .01$) in the 4% but not the 0.5% dose group at all time points in the study.
- There were no significant differences in T_3 levels across treatment conditions.
- There was a significant elevation of rT_3 in the 4% dose group over the 0.5% and control values (p not reported) throughout the duration of the study.

The final segment of this study was an analysis of the morphological changes that the sponsor's consultants found following fixation and preparation of the right lobe of each rat thyroid. There are two reports prepared of the morphological findings; those by the contracting laboratory (Hazleton Laboratories America or HLA) and a later report by Charles C. Capon, D.V.M., Ph.D. of the Ohio State University. In the HLA report of anatomical pathology, it is stated that no treatment-related lesions were observed in any of the microscopically

examined thyroids. Dr. Capen's report of electron micrographs of these thyroids examined thyroids, however, presents different conclusions.

Dr. Capen concluded that the rat thyroids demonstrated:

- hypertrophy of follicular cells with increased development of secretory organelles that was most evident in the rats fed 4% FD&C Red No. 3.
- reversibility of ultrastructural evidence of thyroid follicular cell stimulation following administration of exogenous T_3 during the 7th month of the study.
- a dose-dependent accumulation of numerous, highly irregular, lysosome-like bodies in follicular cells in rats fed FD&C Red No. 3.
- similar changes were found in the lower dose groups (0.25% and 0.5%), but were less well developed.
- similar ultrastructural changes were found in the female rats, but they were considered by the investigator to be less well developed compared to the male rats.

The Division of Pathology (DP) reviewed the electron photomicrographs used by Dr. Capen to derive these findings and derived a somewhat different interpretation of the follicular cell morphology (B.A. Jackson to M. Mack, January 13, 1986). DP concluded:

"The morphological evidence of hypertrophy of follicular cells with increased development of secretory organelles in rats fed FD&C Red No. 3 was not substantiated by the electron micrographs submitted. (Hypertrophy of follicular cells would be better evaluated by light microscopy than by electron microscopy.) Comparisons of the electron micrographs of thyroid follicular cells from male rats that received the plain diet but no T_3 injections and those from rats treated with diets containing 4% FD&C Red No. 3 (but no T_3 injections) revealed no increased development of secretory organelles that could be ascribed to treatment. Within both groups there were differences from cell-to-cell in the relative amounts of rough-surfaced endoplasmic reticulum, Golgi complex, colloid droplets and number and size of microvilli at the luminal surface. There were no differences that characterized one group more so than another. On the other hand, the presence of irregularly-shaped, dense bodies in the cytoplasm of many of the follicular cells of rats that received FD&C Red No. 3 was a conspicuous difference from controls."

DP also concluded that there was little difference in the degree of change to cellular cytoplasmic and rough-surfaced endoplasmic reticulum areas between control males and 4% FD&C Red No. 3 treated males. However, the irregularly shaped, electron dense bodies reported for the

treated males remained in spite of treatment with T_3 . The final overall conclusion by DP was:

"The evidence from the electron microscopic studies does not support the proposed secondary mechanism for the occurrence of thyroid neoplasms but rather it may suggest, on the basis of the lysosomal response, a direct form of injury."

On January 5, 1988, Dr. Charles Capen submitted to CFSAN an analysis entitled, "Final Report: Ultrastructural Histomorphometric Evaluation of Thyroid Glands from Male and Female Rats Fed 0.5% and 4.0% Red No. 3." This analysis was taken from the electron photomicrographs of rat thyroids in control, 0.5% and 4.0% treatment groups from the 6-Month Feeding Study conducted by Hazleton Laboratories America (Project No. 6145-101).

Dr. Capen interpreted these histomorphometric data as indicating that the 0.5% and 4% groups of male rats showed evidence that their thyroid follicular cells were stimulated chronically to a mild to moderate degree by an increased secretion of TSH. Changes that were listed as supporting these conclusions were: increased total cytoplasmic area, increased mean and percent cytoplasmic area occupied by lysosomes, and increased mean size and number of lysosomes in follicular cells compared to control rats. Female rats showed similar changes; however, the changes were not large enough to result in statistically significant (presumably $p > .05$) results.

DP's analysis and evaluation of these data (B. A. Jackson to D. L. Aub, August 1, 1988) agreed that by quantitatively measuring the thyroid subcellular organelles, Dr. Capen had demonstrated evidence of hypertrophy in these rat thyroids providing that the sample selection was appropriate. The overall evaluation of the meaning of these data was:

"Thus, the results, insofar as they provide morphological evidence of functional stimulation of the thyroid gland, are not inconsistent with a secondary mechanism of carcinogenesis in rats. It would seem that the morphological evidence from the ultrastructural studies is insufficient for demonstrating the secondary mechanism of thyroid neoplasia in rats fed diets containing FD&C Red No. 3. While these short term studies can provide evidence suggestive of one or another mechanism, the establishment of mechanism would require longterm studies which demonstrate either enhancement or elimination of carcinogenic effect by modifying components of a putative mechanism."

Witorsch et al. 3-Week Feeding Study

CCMA submitted to the FDA in November 1984 a paper entitled, "Effects of Dietary FD&C Red No. 3 on the Pituitary-Thyroid Axis of Adult Male Rats." This study was performed by R.J. Witorsch at the Medical College of Virginia, Richmond, Virginia. The objective of this study was to

provide additional support for CGMA's hypothesis that FD&C Red No. 3's carcinogenic action on the rat thyroid is mediated through a secondary mechanism. The sponsors hoped to show that exposure to FD&C Red No. 3 would alter the responsiveness of the pituitary *in vivo* to TRH and thereby demonstrate that the dye raised serum TSH levels.

Experimental subjects were male Sprague-Dawley rats obtained from the Charles River Laboratories, Inc. (Wilmington, Massachusetts). Thirteen animals were randomly assigned to the test treatments listed below: control, 0.5%, 1%, 4% FD&C Red No. 3, NaI (100 mg/kg/day) and fluorescein (1.6%) or 1000 mg/kg day). Animals were maintained on Purina Certified Rodent Chow No. 5002 admixed with the various test substances. Rats were housed according to a 12 hour light/12 hour dark cycle. The animals received the test diet for 3 weeks. Near the end of the scheduled feeding period, the rats were shipped by air express to the laboratory of Dr. Anthony Jennings at the University of Pennsylvania for the TRH provocative test.

Thyroid function tests (hormonal assays) were conducted in duplicate for each test animal. Serum T_4 and T_3 were measured using previously published radioimmunoassays; T_3 resin uptake was determined using the Corning Magnetic Resin Uptake Kit and serum TSH was assayed by radioimmunoassay from NIADDK kits provided by A. Parlow.

The authors suggest (see DT memorandum, M. van Gemert to B. Jackson, January 22, 1985) that there was an 80% increase in TSH at 10 minutes post-TRH injection in rats fed 4% FD&C Red No. 3 compared to control rats; however, if the calculations are performed using 0 time for each group as its own control, and calculating % of control values for 5 and 10 minutes then there is no significant difference. Calculating the TSH response as a percent of 0 minutes control values takes into account the variations in baseline values of the individual groups. In addition, there is no evidence for the hyper-response of TSH in the 4% FD&C Red No. 3 group to TRH provocation or the percent released over baseline values would also have been increased (Table 8).

A significant decrease in body weight of the rats was reported for the animals receiving 4% FD&C Red No. 3. The authors attributed the weight loss to the decreased caloric value of this particular regimen. It is difficult to evaluate the merit of this explanation because the level of food consumption is not reported. In some of the other studies (FRI, 6-month and Hazleton, 7-month), animals in the 4% FD&C Red No. 3 treatment groups lost weight in spite of increased food consumption. Thus, this particular result is consistent with those reported from other short-term studies of FD&C Red No. 3.

Following performance of radioimmunoassays, it was found that both serum T_4 and T_3 levels were elevated over control values (Table 9). The increase in T_3 levels is also supported by a decreased T_3 resin uptake, indicating a significant amount of increased endogenous T_3 binding to plasma proteins, which gives a slight but not significantly increased, free T_3 value for the group fed FD&C Red No. 3 at the 4% level. These

findings are not in support of the theory suggested by CCMA that FD&C Red No. 3 blocks the conversion of T_4 to T_3 and thereby disrupts the influence of free T_3 to inhibit TSH release by negative feedback of the pituitary. The data from this study indicate that although there is a very slight increase in serum T_4 levels there is also an increase in serum T_3 in both free and bound forms. This latter finding would not be expected according to the CCMA hypothesis.

A DM memorandum (J.A. Springer to M. van Gemert, January 28, 1986) that critically examined the primary data supplied by CCMA for this paper draw similar conclusions as the aforementioned memorandum by DT (M. van Gemert to B.A. Jackson, January 22, 1985). The DM concluded that there was not a significant effect of FD&C Red No. 3 and TRH on TSH production. Therefore, the hypothesis of any such hyperresponsiveness to TRH, conditioned on baseline measurements, was not demonstrated.

Two Month Hormone Study (Biodynamics)

The CCMA submitted the final report (CAP 96, Vol. 49-52) on this study entitled, "The Secondary Mechanism of Rat Thyroid Oncogenesis: The Results of a Sixty-Day Study of the Effects of FD&C Red No. 3 on Thyroid Hormone Economy in Male Rats" to the CFSAN on January 13, 1989. The study (Project No. 88-3320) was conducted by Biodynamics, Inc. of East Millstone, NJ during the time period August 1, 1986 to September 30, 1988. The objective of this study was to assess the influence of dietary intake of FD&C Red No. 3 on various thyroid and pituitary hormones (TSH, T_4 , T_3 , r T_3) and thyroid histology.

Four hundred eighty CD Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, Michigan) were divided among three study groups (160/group) receiving 0, 0.25% or 4% FD&C Red No. 3 in their daily diets. Twenty rats per test group received FD&C Red No. 3 for 0, 3, 7, 10, 14, 21 or 30 days. The balance of the rats received the test substance for 60 days. Test subjects were placed on test at 3 months of age (young adult) to improve the accuracy (reduce the variability) of the thyroid/pituitary hormone determinations. Animals were allowed ad libitum access to a standard laboratory diet (Purina Certified Rodent Chow No. 5002) and tap water. A 12 hour light/dark cycle (7 A.M. to 7 P. M.) was maintained by an automatic timer. Animals were observed for mortality and gross signs of adverse effects two times daily (at least 5 hours apart).

Twenty randomly selected animals from each treatment group were sacrificed under ketamine anesthesia, exsanguinated and samples taken for thyroid and pituitary glands on Days 0, 3, 7, 10, 14, 21, 30 and 60. Sacrifices were performed from 8AM to 12 Noon. To control for possible influences of diurnal variation on thyroid/pituitary hormone function, sacrifices were rotated (2 rats at a time) through the three treatment groups. Blood samples were obtained from the abdominal aorta and maintained at -20 degrees C. until the end of the study at which time they were shipped to the consultant for radioimmunoassay determination of the effect of FD&C Red No. 3 on serum levels of TSH, T_4 , T_3 and r T_3 .

The only mortality observed during the study was in the 4% dose group. During the second week of the study, three males died spontaneously. While no definitive reason for the deaths of these rats could be determined on necropsy, the fact that all 3 animals underwent pronounced weight loss during the first week of the study led the principal investigators to speculate that the animals died secondary to failure to eat the dietary ration admixed with high levels of FD&C Red No. 3 (4%).

The body weights and rate of body weight gain were equivalent for the 0 and 0.25% treatment groups throughout the study (DM memorandum, C. Barton to C. Bailey, February 23, 1989). The 4% treatment group, however, suffered a pronounced weight loss the first week (approximately 5% of their total body weight) and did not begin to gain weight as a group until the third week of the study. This statistically significant difference in weight compared to controls continued throughout the study and ranged from 13% less at Week 1 to 17% less at Week 8. Weight loss was predictable in the early part of the study because, for example, the 4% group ate 43% less diet for the first week of the study than controls. In addition, greater dietary spillage was observed for the 4% animals for the duration of the study.

The absolute thyroid/parathyroid and pituitary weights of the 4% group were significantly lower than controls at Days 7, 10, 14, 21 and 60 and for all sacrifice periods, respectively. The principal investigators interpreted this decrease to be due to the lowered body weights of the 4% animals compared to controls. The relative thyroid/parathyroid weights were greater at only one sacrifice period, Day 21 and even at this sacrifice interval, the absolute and relative thyroid/parathyroid weights were comparable to controls except at the Day 60 sacrifice, when the 0.25% group values were significantly lower than the control group.

The levels of TSH, T_4 , T_3 and rT_3 for each of the three treatment groups were measured by radioimmunoassay at Days 0, 3, 7, 10, 14, 21, 30 and 60. All treatment groups demonstrated an increase in TSH levels compared to baseline values. The 0.25% group was significantly increased over control levels on Days 21, 30 and 60. In the 4% dose group, all time periods (Days 3-60) showed significantly increased TSH levels compared to controls. On Days 3, 7, 10, and 14, the 4% groups TSH levels were significantly increased over those of the 0.25% group.

The 0.25% group showed a slight increase in T_4 that reportedly reached significance on Day, 10 and 14 compared to both baseline and control. In the 4% group, the serum T_4 was significantly elevated at all time points in the study compared to both baseline and control values.

Serum T_3 concentrations in the 0.25% group differed from the control group values at only one time period, Day 30. Concentrations of the 4% group, however, were significantly lower than baseline and control levels at all time periods during the study and were significantly reduced compared to 0.25% group levels on Days 3, 10, 14, 21, 30 and 60.

The 0.25% group had serum rT_3 that were significantly above those of controls on Days 10, 14 and 21. There were very large increases in serum rT_3 concentrations in the 4% group with significant increases compared to controls and 0.25% values at all time periods (Days 3-60).

In the summary submitted by CGMA on this two-month study of the effects of FD&C Red No. 3 on thyroid/pituitary function, the interpretation of the data was that the hormonal levels of the 0.25% group were unaffected by the experimental treatment; however, the analysis of these data by DM (memorandum from C. Barton to C.J. Bailey, February 23, 1989) indicated a different interpretation based on what DM concludes is a more appropriate statistical analysis of the data. This reanalysis supports the conclusion that the 0.25% dose group also clearly causes TSH and rT_3 to be elevated above control values throughout most of the study (Days 10-21 and 60) and that serum T_4 concentrations were significantly increased on Days 10-21 and 60 as well. Serum T_3 levels were also lower than control values, but reached statistical significance at Day 30 only. Thus, while the quantitative responses of the hormones was somewhat different, the qualitative pattern of hormonal effects for the two dose levels, 4% and 0.25%, was quite similar (DT evaluation memorandum, D.G. Mattan to C. Bailey, April 26, 1989).

The final general response that was measured in the two-month study was the histomorphometric changes occurring with FD&C Red No. 3 treatment. Rats fed both 0.25% and 4% FD&C Red No. 3 for 60 days manifested a significantly decreased thyroid follicular diameter compared to age-matched controls on Days 3, 7, 10 and 14. This effect, however, was not consistent throughout the study and by Day 60 the diameter of the thyroid follicles was greater in the 4% group than in the controls. When compared to baseline values, the diameter of the thyroid follicles was decreased significantly in the 0.25% group at Days 14 and 21 and only on Day 21 for the 4% group.

Area of the thyroid colloid generally followed the same response pattern as diameter of the thyroid follicles. Colloid area was decreased significantly after 3 (4%), 7 (0.25%) and 10 days of feeding FD&C Red No. 3 compared to age-matched controls. Again, this effect was not consistent, and by Day 60 the area of colloid in the 4% group was increased compared to controls.

The height of thyroid follicular cells was decreased significantly in rats fed 0.25% and 4% FD&C Red No. 3 on Days 3, 7, 10, 14, 21 (4% only) and 30 compared to age-matched controls. Only at 60 days of feeding the test substance was a significantly increased follicular cell height observed in both 0.25% and 4% test groups.

With respect to a secondary mechanism of carcinogenesis, there are a number of changes that would be predicted to occur. First there should have been an increase in the weights of the thyroids of the treated rats. In this study, neither the 0.25% or 4% dose groups showed this response. In fact, both of these dose treatments demonstrated lower thyroid weights than the control group. Second, these animals should

have displayed increased T₄, TSH, and rT₃, and decreased T₃. This, in general, is what the animals demonstrated. However, unexpectedly, both the 0.25% and 4.0% groups showed this response. Thus, it was not possible to determine a "no observed effect level" (NOEL) from this study.

Finally, the secondary mechanism of thyroid carcinogenesis would predict that the thyroids would display certain morphological characteristics. For example, early on the follicular cells lining the thyroid follicles should be hypertrophied, then the next stage should be some indication of hyperplasia. The histomorphometric analysis supplied by the sponsor's pathology consultant and our DP (memorandum from B.A. Jackson to C.J. Bailey, February 22, 1989) indicated that early in the study the follicle size and colloid area for both the 0.25% and 4% groups were decreased.

With respect to height of follicular cells, cell heights were less than controls from Days 9-30 and greater at Day 60. The reduction in follicle size and colloid area are what would be expected from thyroid activation by TSH stimulation, but the decreased follicular cell height is not expected. Later in the study (Day 60), when increased follicular cell height is observed, one might conclude that TSH stimulation was occurring as predicted by the sponsor's theory but then the follicle size and colloid area are increased. One would not expect to see these latter effects during thyroid activation.

The DP memorandum's (B. A. Jackson to C. Bailey, February 22, 1989) overall assessment of this histomorphometric analysis by the sponsor's consultant was:

"Thus, this study does not yield evidence of consistent changes in thyroid follicular cell morphology indicative of increased serum thyroid stimulating hormone that could explain subsequent proliferative changes of this gland in rats that accompany the prolonged feeding of FD&C Red No. 3."

METABOLISM STUDIES

Rat Liver Study (Ruiz and Ingbar)

CCMA submitted to CFSAN a study entitled, "Effect of Erythrosine on the Metabolism of Thyroxine in Rat Liver" (CAP 96, Administrative File 7, Entry No. 111, pp. 11082-11086). This study was performed by M. Ruiz and S. H. Ingbar from the Beth Israel Hospital and the Harvard Medical School.

Male Sprague-Dawley rats maintained ad libitum on standard laboratory chow and tap water. Liver homogenates from four rats per dose were studied in duplicate. Both FD&C Red No. 3 and fluorescein were dissolved in water and injected i.p. for 2-7 days.

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Two sets of experiments were conducted, one without tissues and the other set utilizing rat liver homogenates. In the first set of experiments without tissues, the tissue-free control showed no $^{125}\text{I-T}_4$ degradation (see DT evaluation memorandum, J. C. Griffiths to C. Bailey, March 3, 1989). A zero-time sample with FD&C Red No. 3 underwent extensive degradation (21%), while FD&C Red No. 3 added to another sample with a longer, but unspecified time of exposure demonstrated marked degradation that was decreased by protection from light or addition of serotonin.

The second set of experiments were performed with rat liver homogenates. In this series of experiments, doses of 2.5, 10, 50 and 250 mg/kg were administered i.p. to the experimental animals for 2-7 days. Next, liver homogenate samples from these animals were used to test for the influence of previously administered FD&C Red No. 3 on the conversion of $^{125}\text{I-T}_4$ to $^{125}\text{I-T}_3$ and ^{125}I . The rate of this conversion was compared with liver homogenates from animals receiving as pretreatments; water, as a vehicle control, or fluorescein (250 mg/kg body weight). There were also zero time and low temperature controls included in the test samples. Only the rats treated with i.p. FD&C Red No. 3 demonstrated a dose-dependent reduction in the rate of conversion of $^{125}\text{I-T}_4$ to $^{125}\text{I-T}_3$ and ^{125}I . The results also indicated that the conversion of T_4 to T_3 proceeds more rapidly than the conversion of T_3 to T_2 .

A number of drawbacks concerning the performance of this study are listed below:

- The authors of the study did not specify whether the dye used was certified or not.
- The route of administration was inappropriate; animals were injected i.p. rather than being exposed to the FD&C Red No. 3 in the diet or through the skin.
- Only summary or derived data were provided and the assumptions for the derived calculations were not given.
- Certain conditions were not stated; for example, in the tissue-free experiments, no information was given on the duration of the experiments, or the concentrations of FD&C Red No. 3 used in the reaction tubes.

While it appears that this study provides some support for the hypothesis that when FD&C Red No. 3 is injected i.p., it is able to inhibit the peripheral (liver and kidney) conversion of T_4 to T_3 in the rat, it is not clear what might happen during usual circumstances that prevail where FD&C Red No. 3 exposure is by mouth or through the skin.

Metabolism Segment of Seven Month Study (Hazleton Labs)

The samples for these metabolic experiments came from rats included in the study (Project No. 6145-101) entitled, "Seven-Month Feeding Study to Assess the Effect of FD&C Red No. 3 on Thyroid Function in Rats" that

was submitted to the NDA on November 5, 1984. The data used to compile this analysis may be found in CAP 96, Vol. 36, pp. 11348-11381. The authors of this paper were S.H. Ingbar, A. Bauman and L.E. Braverman at Beth Israel Hospital and Harvard Medical School (Boston, Massachusetts) and the Dept. of Endocrinology, Univ. of Mass. Medical Center, Worcester, Mass.

The objective of this study was to derive evidence to indicate the mechanism for the stimulation of release of TSH from the rat pituitary following administration of FD&C Red No. 3, and more specifically how the peripheral metabolism of T_4 may influence circulating levels of T_4 and T_3 .

The test compound was FD&C Red No. 3; certified lot no. AC4862. Male Sprague-Dawley rats (CD) from Charles River Breeding Laboratories supplied the test animals. The rats were fed either 0 (*,\$), 0.5 (*), 1.0 (\$), 2.0 (\$) and 4.0 (*,\$)% FD&C Red No. 3 in the diet for 7 months. Animals with a (*) designation were used for liver studies, while those with a (\$) designation were used for pituitary samples. Tissue samples of liver and pituitary were shipped frozen to the laboratory of S.H. Ingbar. Livers and pituitaries were homogenized in phosphate buffer at pH 7.4.

$^{125}I-T_4$ along with dithiothreitol was added to liver or pituitary homogenate in reaction tubes. The mixture was shaken and incubated at 37 degrees C. for 3 hours. Zero time specimens were prepared in which the stop solution was added immediately. Supernatants from the precipitated reaction mixtures were analyzed for ^{125}I -labeled components by descending paper chromatography. Following autoradiography, the radioactive zones were excised and the ^{125}I content measured. The proportion of $^{125}I-T_4$ degraded and the percentage of ^{125}I -labeled products generated were calculated.

No individual animal data was provided, only bar graphs and calculations summarizing the results. Standard deviation bars were not provided to allow one to determine how much variation existed between the five rats in each group. Within the limitations of the data presentation, the degradation of T_4 was decreased to 40% of control values in the liver homogenates of rats fed 4% FD&C Red No. 3. There was also a 75% decrease in the generation of ^{125}I -iodine and a 80% decrease in the generation of $^{125}I-T_3$. The degradation of these components by the low dose rats (0.5%) was similar to that of controls. The high dose (4%) changes were statistically significant ($p < .001$).

The authors reported the availability of only small amounts of pituitary for homogenation. Thus, the samples were required to be diluted 10-fold more than the liver samples. This procedure led to a lower rate of T_4 metabolism in this experiment. Again, no individual animal values were provided, however, the data supplied indicates that in the higher dose groups (2% and 4%), the rate of T_4 degradation and I (iodide ion) generation was higher than controls. There was an increased generation of T_3 only in the 2% dose group; however, the overall trend of the data

suggested increased T_4 degradation and T_3 and I generation. None of the reported changes in the pituitary reached statistical significance.

The conclusion from the DTRE memorandum (J. C. Griffiths to C. Bailey, April 5, 1989) is that the authors of the study apply the *in vitro* data selectively; the liver homogenate data supporting their hypothesis and the "tentative" information from the pituitary homogenate data is not sufficiently explained nor used. The DTRE memorandum concludes that because of the shortcomings of the study described above, the sponsor's hypothesis that FD&C Red No. 3 mediated impairment of T_4 metabolism leads to decreased production of T_3 and the subsequent increased secretion of TSH is not supported. The hypothesis is not supported unequivocally by the liver data because that would require direct assessment of the effects of erythrosine on the metabolic clearance rate of T_4 *in vivo* and does not take into consideration the conflicting pituitary results.

Hazleton ADME Study (Project No. 6145-100)

The CGMA submitted a partial report on this study to the CFSAN on March 4, 1986; the final report was received on February 27, 1989. This study has also been referred to in other documents as "Metabolism of FD&C Red No. 3 in Rats" and "Pharmacokinetics and Biotransformation of FD&C Red No. 3 in Rats." The data is located in GAP 96, Vol. 53 and 54.

FD&C Red No. 3 was of stated chemical (range, 91.7-94.8%) and radiochemical (range, 85.7-95.7%) purity; unlabeled FD&C Red No. 3 came from certified lot no. AC7172. Dosing solutions were made from both the radioisotopically labeled and unlabeled supplies of FD&C Red No. 3.

The Phase A study used Charles River Cr1:CD(SD)BR male rats (Stone Ridge, NY), whereas the Phases I and II used Harlan Sprague-Dawley from Madison, Wisconsin. The rats were about 7 weeks of age when used for the study. Animals were housed individually in stainless steel cages, except when being used for metabolic experiments. During these procedures, the rats were maintained individually in glass metabolism cages.

Table 10 shows the various doses, induction diets, radiolabel identification and number and sex of rats used. All test animals from each of the 3 Phases of testing received either 0, 0.5% or 4.0% FD&C Red No. 3 for 7-8 days before receiving the radiolabeled compounds. The Phase A (preliminary study) used 14 males/radiolabel/group and were given 0.2 g of each of two radiolabeled compounds/kg body weight (b.w.) (either the ^{125}I or ^{14}C). The Phase I animals (Preliminary Excretion Balance) animals, 2 males and 2 females/radiolabel/group, were given 0.2 g of each of the radiolabeled compounds per kg b.w. Phase II (Absorption, Distribution and Excretion) control animals (48 males and 48 females/radiolabel/group) received either 0.2 or 1.0 g of either ^{14}C or ^{125}I test compound/kg b.w. The two groups pre-exposed to FD&C Red No. 3 (0.5% and 4.0%) received 0.2 g of either of the two radiolabeled test compounds/kg b.w.

Sample collection was carried out as indicated below:

- Phase A: 2 rats/radiolabel/group were sacrificed predose, and 1, 2, 4, 8, 24 and 48 hours post-dosing. Blood, urine and fecal samples were collected.
- Phase I: 2 rats/radiolabel/group were housed in metabolism chambers and the concentrations of CO₂ and other volatiles were determined at predose, 0-4, 4-8, 8-24, and 24-48 hours after dosing. Urine and feces were collected every 24 hours out to 168 hours postdosing.
- Phase II: 4 rats/radiolabel/group were sacrificed and blood samples collected at predose, 1, 2, 4, 8, 24 and 48 hours postdosing. Urine and feces were collected according to the schedule listed under Phase I above.

Observations of physical changes due to consumption of FD&C Red No. 3 were restricted to occurrence of pink to red hair and feces (DTRE memorandum from J. C. Griffiths to C. Bailey, April 3, 1989). Some of the animals from the various 4% groups had lower body weights than animals that did not receive FD&C Red No. 3 during the study. Some of the animals from both the 0.5% and 4.0% groups consumed more food than the controls. There were sporadic instances of decreases in liver, kidney and thyroid weights; however, there did not appear to be a systematic trend to these events as the changes occurred irregularly in both control and FD&C Red No. 3 treated animals.

The conclusions of the findings from this study are included in the DTRE memorandum of April 3, 1989 (J. C. Griffiths to C. Bailey).

" This study demonstrates that the majority of the orally administered test compound is excreted unchanged in the feces. Approximately 75% of the administered radioactivity was recovered in the feces by 48 hours, and unchanged erythrosine accounted for approximately 85% of the total radioactivity in the feces. The minor amounts absorbed are metabolized, with diiodo- and triiodofluorescein appearing in the urine, plasma, liver and kidney. Up to 25% of the labeled test compound was not found in the feces, and was therefore absorbed. From the absorbed erythrosine, the percent remaining unchanged was very low in the urine and kidney (1-10%) and up to 50% in the plasma and liver. The remainder was divided into diiodo-and triiodofluorescein metabolites. The thyroid only sequestered ¹²⁵I, however, since the ¹⁴C label did not concentrate within this tissue, the hypothesis that the ¹²⁵I label represents free iodine and not a FD&C Red No. 3 product is plausible."

SUMMARY AND CONCLUSIONS

The sponsors of FD&C Red No. 3 have developed an hypothesis relating the thyroid tumorigenesis resulting from longterm exposure to FD&C Red No. 3 by attributing the neoplastic process to an indirect effect of

alteration of the normal thyroid/pituitary hormonal interactions. The sponsors postulate that the presence of FD&C Red No. 3 in the rat blocks the peripheral conversion of T_4 to T_3 by the inhibition of a liver and kidney enzyme, 5'-monodeiodinase. The resulting depression of T_3 levels and lack of negative feedback on the pituitary leads to a tonic and protracted increase in the secretion of TSH that in turn mediates continuous overstimulation of the thyroid follicular cells and the eventual expression of tumors (follicular adenomas). In a general sense, this secondary mechanism is quite similar to that suggested to cause thyroid tumors in rats exposed to sulfonamide-type agents.

Compared to some of the sulfonamide-type agents, however, FD&C Red No. 3 is a weak goitrogen. A number of the sulfonamides are potent inhibitors of thyroid peroxidase in the thyroid gland. This interference with thyroid hormone production (T_4 and T_3) at two separate steps in their synthesis leads to a virtual blockade of thyroid hormone production. With this large reduction in circulating thyroid hormones, the pituitary is stimulated to produce and release unusually high and continuous amounts of TSH due to the lack of negative feedback on the pituitary normally provided by the thyroid hormones.

In the case of FD&C Red No. 3 the sponsors theorize, based on the experimental data that they have provided (pharmacokinetic, metabolic and thyroid hormone data), that FD&C Red No. 3 inhibits an enzyme in the liver and kidneys, 5'-monodeiodinase. This, it is suggested, leads to the increased levels of TSH, T_4 and rT_3 , and decreased levels of T_3 that were demonstrated in the two-month study conducted by Biodynamics. According to the sponsors theory, however, there should be a tonic secretion of excess amounts of TSH that continuously stimulates the thyroid follicular cells.

The two studies conducted to gather data covering an intermediate time period (six to seven months following initiation of exposure to FD&C Red No. 3) demonstrate poor to equivocal evidence to support this hypothesis of continuing hypersecretion of TSH. Only in the PRI Six-Month study is there evidence for significantly elevated levels of TSH (and then only in male rats in their "in-life" phase analyses, and not in their "serial" phase analyses). This finding is also equivocal because of the lack of control for diurnal variation in this study. There is no evidence for increased TSH secretion in the Hazleton seven-month study or the Witorsch three week study. Some of the other hormonal data provided by these studies do support the findings of the two-month Biodynamics study. In the PRI study, both the "in-life" and "serial" phases clearly indicate an increased level of T_4 in the 4% male rats. Also, the "in-life" phase for male rats indicates decreased T_3 compared to the controls; however, the "serial" phase does not show the same results regarding T_3 levels. The Hazleton seven-month study demonstrates an increased level of T_4 and rT_3 with decreased levels of T_3 . The Witorsch three week study, however, shows increased levels of T_4 as would be predicted by the sponsor's model, but inappropriately shows increased not decreased levels of T_3 .

Along with the prediction of long-term elevation of TSH secretion by the pituitary caused by depressed levels of T_3 , the sponsor also hypothesize that the thyroid gland should manifest evidence of stimulation by the excessive levels of TSH. This stimulation should be demonstrable by certain morphological changes of the thyroid. Across time the thyroid should show evidence of hypertrophy, hyperplasia, adenomas and perhaps carcinomas. Thus, it should be possible to discern a pattern of progressive change in the proliferative lesions present in the thyroid glands of animals continuously exposed to FD&C Red No. 3.

In the two-month study performed by Biodynamics, the morphometric changes which were observed could best be characterized as paradoxical or at least inconclusive. The thyroids of FD&C Red No. 3 exposed animals apparently did show some early evidence of activation by the TSH (follicle size and colloid area were decreased early); however, the cells lining the follicles were decreased in size instead of hypertrophied. Later on in the study (Day 60), the follicular cells did become larger than those of the control animals, but then the other signs (follicle size and colloid area) became larger, events that are not consistent with the sponsor's model.

Although the sponsor's consultant interpreted the electron micrographs from the Hazleton seven-month study and the PRI six-month study to be consistent with hypertrophy of the thyroid follicular cells, this was not apparent from the electron micrographs provided. The level of subcellular development by both the control group and 4% FD&C Red No. 3 animals appeared to be similar. It was only when the sponsor's consultant went to the unusual lengths of quantitatively measuring the cells did DP agree that hypertrophy had been demonstrated. An unresolved question or concern remains, however, of how the regions for the electron micrographs were chosen. It would be difficult to assure that the regions chosen were representative of each thyroid gland as a whole for either the control or the FD&C Red No. 3-treated groups.

The final discussion regarding morphological change relates to FD&C Red No. 3 induced thyroid gland alterations in the IRDC chronic study. The results of the terminal sacrifice are agreed on by both the sponsor and CFSAN. In this case there was a clear increase in the incidence of follicular cell adenomas of male rats in the 4% FD&C Red No. 3 group compared to their incidence in male control animals; 21% and 1.5%, respectively. According to the sponsor's theory, there should also be an increased incidence of proliferative lesions in the 4% group of animals compared to controls at the one-year interim sacrifice. The sponsor's consultant concluded that these animals demonstrated hyperplasia (9 of 10 animals). DP, however, in an earlier analysis of the same slides, did not diagnose hyperplasia in these 4% FD&C Red No. 3 exposed, interim sacrificed animals.

While it would seem important for the sponsor to establish some sort of progression in proliferative lesions to support their hypothesis of continuous overstimulation of the thyroids by TSH, it is also conceivable that regardless of what the actual carcinogenic mechanism

might be; i.e., direct or indirect, that hyperplasia would be expected in the thyroids after a year of treatment.

Another aspect of the Hasleton seven-month study is relevant to the discussion of possible mechanism of carcinogenesis; i.e., the attempt to demonstrate reversibility of the changes induced by FD&C Red No. 3. In this particular case however, the only proliferative change that the sponsor was able to show was hypertrophy. Even if one agrees that the rat thyroids regressed toward "normality" in the presence of the supraphysiological doses of T₃ administered, the expression of hypertrophy in endocrine glands is also associated with increased levels of normal activity. Thus, the sponsors have not demonstrated hormonally-based reversal of proliferative changes, such as clearly manifested hyperplasia, that could be more easily defended as being associated with progression to tumors.

A final issue of direct interest and relevance to the determination of the possible mechanism underlying the carcinogenicity of FD&C Red No. 3, is whether FD&C Red No. 3 is genotoxic. While it is true that FD&C Red No. 3 does not appear to be mutagenic in well-accepted test systems, there are still unresolved questions regarding its potential to interact with and damage genetic material as indicated by other assays with cytogenetic endpoints. Studies carried out *in vitro* show that there is 1) a weak positive effect for induction of chromosomal aberrations in CML cells and 2) a positive response for induction of micronuclei in V79 cells. Further, there are two independent *in vivo* studies on micronuclei formation in which there are increased micronuclei frequencies (3-fold to 8-fold) in mice treated with the color. Although statistical analysis was inadequate for both studies, there is an indication of a positive response supported further by the fact that in one of the studies the increased frequencies were dose related.

In summary, while the sponsors of FD&C Red No. 3 have amassed certain evidence that supports their theory of the mechanism of action of this compound; e.g., the inhibition of 5'-monodeiodinase, the attendant thyroid/pituitary effects early after the initiation of exposure to FD&C Red No. 3; there are inadequacies in other aspects of their support for their hypothesis of an indirect mechanism for FD&C Red No. 3's thyroid tumorigenesis. For example, they have not adequately supported their contention that TSH is secreted in higher than normal levels for essentially the full duration of the rat's life. Moreover, the sponsors have not successfully demonstrated the full course of morphological events that are necessary to support their contention that the presence of TSH mediates a series of progressive proliferative lesions of the thyroid leading to the expression of thyroid tumors.

With the data provided thus far by the sponsors of FD&C Red No. 3, it is equally feasible (as an alternative to their hypothesis of an indirect effect of FD&C Red No. 3) to interpret their test results as indicating an early hormonally-mediated effect on the rat thyroid, followed by thyroid compensation and a subsequent return of the gland to a normal

hormonally responsive state and much later by the expression of thyroid tumors in a separate series of events that are unrelated to the hormonal perturbations shown by the rat thyroid early on in its exposure to FD&C Red No. 3. This latter scenario would assume that the final occurrence of tumors was due to a primary carcinogenic effect of FD&C Red No. 3 independent of its ability to mediate hormonal changes of the thyroid/pituitary axis.

David G. Hattan, Ph.D.

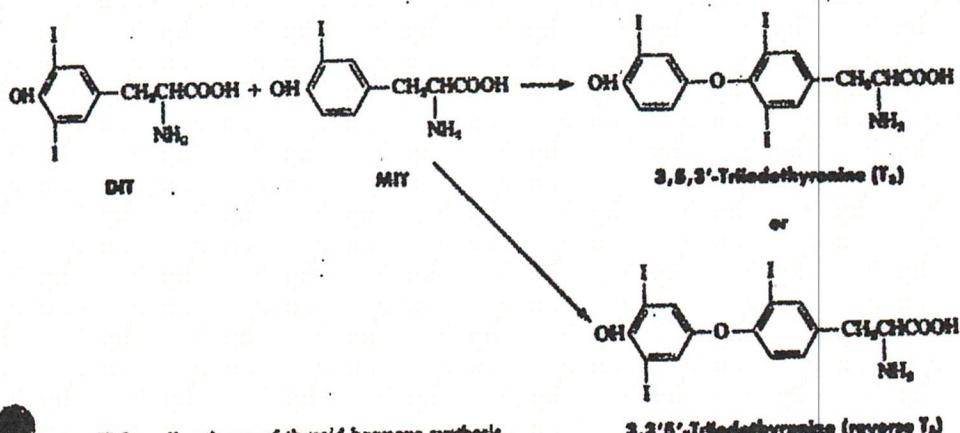
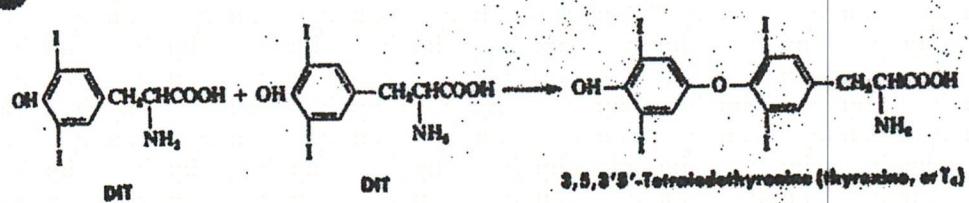
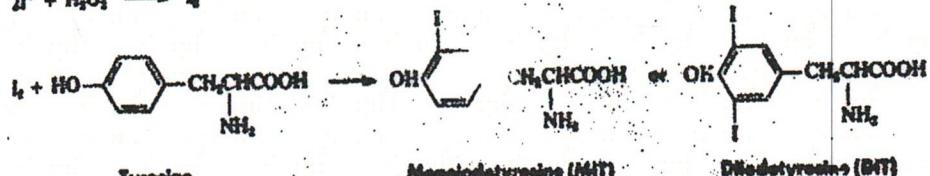
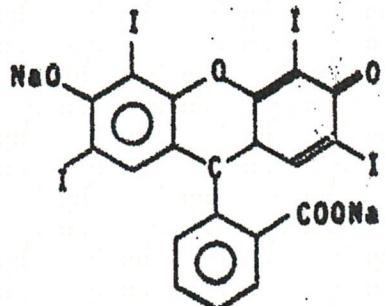
Attachment: Tables

HFF-100
HFF-152
HFF-156 (Edwards)
HFF-158 (Riddle, Taylor)
HFF-334
HFF-445

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FIGURE 1 - Structural Formula - FD&C RED NO. 3 (ERYTHROSINE);



2 ■ Overall pathway of thyroid hormone synthesis.

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Table I
FD & C Red No. 3

Thyroid Follicular Proliferative Lesions in Male Rats
According to the Division of Pathology, Bureau of Foods'
Evaluation of Thyroid Slides
(Pathology Petition Review No. PR-97)

<u>Groups*</u>	<u>Follicular Hyperplasia (%)</u>	<u>Follicular Adenoma (%)</u>	<u>Follicular Carcinoma (%)</u>	<u>Follicular Adenoma + Follicular Carcinoma (Combined) (%)</u>
IA (control)	1/65 (1.5)	0/65 (0.0)	0/65 (0.0)	0/65 (0.0)
IB (control)	0/61 (0.0)	0/61 (0.0)	1/61 (1.6)	1/61 (1.6)
II (0.1% FD&C Red No.3)	7/64 (10.9)	3/64 (4.7)	3/64 (4.7)	6/64 (9.4)
III (0.5% FD&C Red No.3)	2/66 (3.0)	7/66 (10.6)	1/66 (1.5)	8/66 (12.1)
IV (1.0% FD&C Red No.3)	3/57 (5.3)	1/57 (1.8)	3/57 (5.3)	4/57 (7.0)

*IRDC Study No. 410-002

↑
Correct

Correct as reported
by Borzelleca

Table 2
Thyroid Follicular Proliferative Lesions in Female Rats
According to Dr. Capen's Evaluation

<u>Groups</u>	<u>Cystic Follicular Hyperplasia</u>	<u>Follicular Cell Adenoma</u>	<u>Follicular Cell Carcinoma</u>
<u>IRDC</u>	(%)	(%)	(%)
<u>Study No. 410-002</u>			
IA Control	1/70 (1.4)	1/70 (1.4)	0/70 (0)
IB Control	1/68 (1.5)	0/68 (0)	0/68 (0)
II (0.1% FD&C Red No. 3)	3/68 (4.4)	1/68 (1.5)	0/68 (0)
III (0.5% FD&C Red No. 3)	3/67 (7.5)	3/67 (4.5)	0/67 (0)
IV (1.0% FD&C Red No. 3)	4/68 (5.9)	5/68 (7.4)	^a 1/68 (1.5)
<u>IRDC</u>			
<u>Study No. 410-011</u>			
IC Control	4/66 (6.1)	0/66 (0)	0/66 (0)
V (4.0% FD&C Red No. 3)	5/68 (7.4)	5/68 (7.4)	0/68 (0)

^aOne (1) animal (58020XA) had a cystic follicular hyperplasia and follicular cell carcinoma.

↑
Correct
as per
Buzellecs

019982

Table 3.1 Individual Organ Weights - Technical Specimens

Group, Rat Number	Sex	Body Wt. g	Bladder g	Liver g	Kidney g	Testes/ Ovaries g	Brain g	Thyroid mg
<u>02 (Consecutive)</u>								
91013XJ	M	577	0.85	27.30	6.35	3.34	2.41	45
91013XK	H	614	0.76	22.15	6.31	2.09	2.22	54
91020XR	M	*	1.05	21.65	4.16	2.65	1.17	37
91029XJ	M	593	0.99	21.07	4.30	2.95	2.22	51
91032XJ	M	734	1.31	29.90	6.47	1.85	2.37	39
91033XK	H	843	0.92	20.33	5.05	3.75	2.19	51
91035XJ	M	600	1.19	18.56	4.14	3.87	2.00	41
91037XJ	M	531	0.93	20.94	4.38	3.88	2.07	36
91038XR	M	693	5.48	29.34	5.79	3.51	2.30	32
91064XK	M	549	1.17	18.63	4.72	2.97	2.20	42
91053XJ	H	627	1.00	23.40	6.35	3.75	2.10	45
91057XJ	M	508	1.09	18.09	4.18	4.10	2.21	46
91007XB	P	410	0.60	17.95	3.13	.91	1.92	30
91015XA	P	686	1.02	21.08	3.24	143	2.12	36
91017XB	P	425	0.71	14.18	3.34	136	1.77	37
91025XA	P	605	1.28	22.67	3.00	176	2.07	57
91026XA	P	551	0.33	14.43	2.97	113	1.92	28
91027XA	P	673	0.61	29.92	4.21	.99	1.97	46
91029XA	P	484	0.61	13.77	3.35	136	2.07	41
91034XA	P	486	1.02	15.87	5.38	170	2.13	25
91036XA	P	358	0.35	15.24	3.06	133	2.11	62
91044XA	P	416	0.61	14.23	3.28	173	2.08	50
91045XA	P	335	1.11	16.55	3.76	.43	2.20	28
91047XA	P	425	0.58	14.26	2.68	147	2.07	50

From: CAP 96, Vol. 25

$\bar{x} = 43.25$
 Range = 32 - 54

*Inadvertently set recorded

410-011

01S983

HG

405743

TABLE 4 Individual Organ Weights, Formalin Fixation

Group, Rat Number	Sex	Body Wt. g	Spleen g	Liver g	Kidneys g	Trachea/ Ovaries g	Brain g	Thyroid g
<u>All:</u>								
91129XJ	M	485	0.78	15.50	4.75	2.77	2.02	49
91133XJ	M	523	1.03	19.57	5.04	3.71	2.17	76
91137XJ	M	726	1.16	20.01	4.30	6.46	2.11	50
91140XJ	M	689	1.43	16.61	4.62	2.71	2.34	47
91142XJ	M	447	0.84	21.24	5.14	2.85	2.19	43
91143XJ	M	516	1.33	23.16	4.23	8.65	2.30	57
91143XK	M	534	0.46	23.44	4.44	4.63	2.16	46
91146XK	M	525	0.93	16.91	6.15	3.73	2.33	109
91149XJ	M	442	1.79	19.05	4.33	2.30	2.16	35
91149XK	M	557	1.08	16.95	4.47	3.19	2.20	530
91158XJ	M	602	1.00	21.40	4.57	3.30	2.00	67
91159XK	M	536	0.81	18.72	4.50	7.57	2.30	48
91160XJ	M	732	1.29	26.95	5.94	3.48	2.24	50
91165XJ	M	589	0.91	23.30	5.62	3.90	2.30	316
91167XJ	M	567	0.70	21.56	4.57	3.74	2.03	37
91168XK	M	454	0.74	18.28	4.39	3.27	2.27	43
91171XK	M	660	1.25	24.14	5.40	7.45	2.43	45
91173XK	M	449	0.85	27.46	6.85	2.46	2.02	90
91175XJ	M	490	1.30	20.82	5.39	3.00	2.27	43
91177XJ	M	637	0.35	20.82	4.94	3.34	2.66	54
91184XK	M	462	1.20	26.96	4.17	10.06	2.09	111
91185XJ	M	665	1.11	25.09	6.13	3.63	2.27	64
91185XK	M	513	0.92	16.23	4.61	2.70	2.17	56
91126XA	F	404	0.40	19.04	3.04	1.64	2.00	31
91129XA	F	349	0.48	15.17	2.73	99	1.78	25
91133XA	F	349	0.84	13.82	3.99	82	1.85	41
91139XA	F	401	0.51	13.07	3.49	104	1.58	41
91142XA	F	274	0.72	9.95	2.48	79	1.67	36
91142XB	F	271	0.98	12.76	3.17	99	1.81	39
91143XA	F	329	0.51	23.37	3.30	94	2.13	32
91143XB	F	377	0.66	19.37	3.92	169	2.09	36
91144XA	F	361	0.55	11.63	2.67	93	1.99	49
91144XB	F	344	0.59	13.42	2.85	108	1.90	71
91147XA	F	406	0.90	22.14	3.72	79	2.03	49
91147XB	F	640	1.06	18.09	3.73	89	1.87	94
91149XB	F	456	0.83	16.27	2.81	169	2.16	36
91150XA	F	242	0.36	11.62	3.22	39	2.17	31
91150XB	F	352	2.11	13.03	3.45	67	2.09	27
91151XA	F	439	0.97	13.92	3.62	138	1.94	39
91158XA	F	337	0.88	10.63	2.72	178	1.95	38
91165XA	F	442	0.74	15.53	3.70	130	2.19	45
91167XB	F	331	0.77	13.36	3.62	104	2.08	35
91169XA	F	400	2.27	16.05	2.87	63	1.85	18
91171XA	F	363	0.37	13.87	3.25	90	2.14	51
91177XB	F	347	0.64	19.30	3.59	232	1.88	37
91184XA	F	356	0.64	15.39	3.00	132	2.07	46
91184XB	F	290	0.73	14.98	2.77	130	2.00	23

From: CAP 96, Vol. 25

$$\mu = 91.83$$

212%↑

Range = 37-558

- Frontiers (558 & 314)

$$\mu = 59.05$$

136%

Range = 37-111

019984

Table 5
Thyroid Neoplasias in IRDG Study No. 410-011

	Dose	Males		Females	
		0%	4%	0%	4%
<u>Thyroid</u>					
No. examined	59	58		56	59
-C Cell adenoma	3	8		2	3
-C cell carcinoma	1	2	f	0	0
Total	4	10		2	3
- Follicular carcinoma	1	3	R	0	1
- Follicular adenoma	0	17	R	0	2
Total	1	18*	R	0	3
<u>Liver</u>					
No. examined	59	58		57	57
- Neoplastic nodule	4	3		2	5
- Ga. carcinoma	0	0		0	3
Total	4	3		2	8
<u>Adrenal</u>					
No. Examined	59	58		57	58
- Pheochromocytoma adenoma	1	6		0	0
<u>Testes</u> - No. examined	60	58		-	-
- Interst cell adenoma	4	11		-	-
<u>Uterus</u>					
- Polyp	-	-		57	58
	-	-		1	4

2/18/20
7:00 AM
Corresponding to 3 at
Reported by
Buzelle,
but only
1 in control
whereas
Buzelle

Table 6

Male Rats with Thyroid Follicular/C - cell Lesions
According to the Division of Pathology, Bureau of Foods'
Evaluation of Thyroid Slides

<u>Groups</u>	<u>Follicular Hypertrophy</u> (%)	<u>Follicular Adenoma</u> (%)	<u>Follicular Carcinoma</u> (%)	<u>Follicular Adenoma + Follicular Carcinoma (Combined)</u> (%)
I (Control)	4/68 (5.9)	1/68 (1.5)	1/68 (1.5)	2/68 (2.9)
II (4% FD&C Red No. 3)	11/68 (16.2)	14/68 (20.6)	5/68 (7.4)	18 ^a /68 (26.5)
				correct as KpnH D1 CS1
<u>C - cell Hyperplasia</u> (%)	<u>C - cell Adenoma</u> (%)	<u>C - cell Carcinoma</u> (%)	<u>C - cell Adenoma + C - cell Carcinoma Combined</u> (%)	
I (Control)	5/68 (7.4)	4/68 (5.9)	1/68 (1.5)	5/68 (7.4)
II (4.0% FD&C Red No. 3)	9/68 (13.2)	5/68 (7.4)	4/68 (5.9)	9/68 (13.2)

^aIn combining the incidence of animals with follicular cell tumors, one animal with both adenoma and carcinoma was counted as one (1).

013986

Table 7: Histopathological Diagnoses of Selected Animals from
4.0% EBBC, Rad. No. 1

Animal Number	Findings	Day of Death
<u>Initiation - interim sacrifice</u>		
91152XX	F.C. hyperplasia (++)	D196
<u>Interim Sacrifice (at 1 year)</u>		
91129XX	F.C. hyperplasia (+++)	S364
91144XX	F.C. hyperplasia (++)	S364
91147XX	F.C. hyperplasia (+)	S364
91148XJ	F.C. hyperplasia (++)	S364
91150XX	F.C. hyperplasia (++)	S364
91151XJ	F.C. hyperplasia (++)	S364
91151XX	F.C. hyperplasia (+++)	S364
91167XX	F.C. hyperplasia (++)	S364
91172XX	F.C. hyperplasia (+++)	S364
91184XJ	F.C. hyperplasia (++)	S364
<u>Interim Sacrifice - terminal sacrifice (days 365-875)</u>		
91173XJ	F.C. hypertrophy (+)	E371
91128XX	F.C. (+), follicular cyst	D504
91141XJ	F.C. hypertrophy (+)	D567
91160XX	F.C. hyperplasia (++) follicular cystic hyperplasia	E616
91152XJ	F.C. hyperplasia (++)	E637
91145XX	F.C. hypertrophy (+), follicular cystic hyperplasia	D665

03/28/89

Table 8

Effects of FD&C Red No. 3, Fluorescein and Sodium Iodide
on Basal and TRH Induced Serum TSH Levels in Adult Male Rats.

Treatment	No. of rats	Serum TSH (ng/ml) ± S.E.			As % of 0 min. control	
		0 min.	5 min.	10 min.	5 min.	10 min.
1) Control	10	3.4±0.7	12.3±1.3	12.2±1.2	361.8	358.8
2) Red 3, 0.5%	12	5.3±1.4	16.0±3.5	17.0±2.6	301.9	320.8
3) Red 3, 1.0%	12	4.3±0.7	13.9±3.5	15.6±1.4	323.2	362.8
4) Red 3, 4.0%	12	7.0±1.1	18.2±2.3	22.0±3.1	260.0	314.3
5) NaI	12	4.6±0.7	14.1±1.6	16.2±2.0	306.5	352.2
6) fluorescein	12	4.5±1.0	13.1±1.3	14.1±1.2	291.1	313.3

019988

Table 9

Effect of FD&C Red Dye No. 3, Fluorescein and Sodium Iodide on Body Weight, Serum T4 and T3 Levels and T3 Resin Uptakes in Adult Male Rats

Treatment	Body wt. g	Serum T4 ug/dl	Serum T3 ng/dl	T3 RU ^f	FT4I ^g	FT3I ^h
1. Control (11)	340±10	3.76±0.12	44±3 ^a	48.7±0.5	1.63±0.05	21.2±1.4
2. RD 3, 0.5% (12)	348±7	4.11±0.14	42±1	49.0±0.6	2.01±0.05	20.5±1.4
3. RD 3, 1.0% (12)	357±8	4.66±0.17 ^b	45±1	48.6±0.6	2.27±0.07 ^b	21.7±0.7
4. RD 3, 4.0% (12)	308±6 ^a	3.05±0.15 ^c	52±3 ^e	43.2±0.5 ^a	2.18±0.14 ^d	22.6±1.4
5. NaI (12)	350±5	4.57±0.23 ^d	46±3	48.2±0.6	2.19±0.10 ^d	21.9±1.2
6. Fluorescein (12)	355±8	3.73±0.16	43±2	49.8±0.6	1.85±0.07	21.1±1.0

Numbers in parentheses indicate number of rats per treatment
Each value represents mean ± SE.

^ap<0.01 vs all treatments.

^bp<0.01 vs 1, 6; P < 0.05 vs 2.

^cp<0.01 vs 1, 2 and 6.

^dp<0.01 vs 1, 6.

^ep<0.05 vs 1, 2, 6.

^fT3 resin uptakes in per cent.

^gCalculated as product of serum T4 and T3 resin uptake.

^hCalculated as product of serum T3 and T3 resin uptake.

012989

Table 10
Summary of Radicelabeled Dosing, Induction Diet and Sample Size
in the Pharmacokinetic/Metabolism Study by Hasleton Laboratories

<u>FDG Red (Mo. 3 induction diet</u>	<u>Radicelabeled test compound</u>	<u>Radiclabel</u>	<u>Number animals/sex</u>
<u>PHASE A</u>			
0 %	0.2 g/kg	^{14}C ^{125}I	14 14
0.5 %	0.2 g/kg	^{14}C ^{125}I	14 14
4.0 %	0.2 g/kg	^{14}C ^{125}I	14 14
<u>PHASE I</u>			
0 %	0.2 g/kg	^{14}C ^{125}I	2 2
0.5 %	0.2 g/kg	^{14}C ^{125}I	2 2
4.0 %	0.2 g/kg	^{14}C ^{125}I	2 2
<u>PHASE II</u>			
0 %	1.0 g/kg	^{14}C ^{125}I	48 48
0 %	0.2 g/kg	^{14}C ^{125}I	48 48
0.5 %	0.2 g/kg	^{14}C ^{125}I	48 48
4.0 %	0.2 g/kg	^{14}C ^{125}I	48 48

01S990